Saskatchewan Ministry of Agriculture

FINAL REPORT

RESEARCH

ADF

AGRICULTURE DEVELOPMENT FUND

20050732

STRATEGIES TO MODIFY CONNECTIVE TISSUE AND ENHANCE TENDERNESS OF MATURE BEEF

Funded by: The Agriculture Development Fund

November 2010

Prepared by: University of Saskatchewan (U of S)

UNIVERSITY OF SASKATCHEWAN

College of Agriculture and Bioresources

Strategies to Modify Connective Tissue and Enhance Tenderness of Mature Beef

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Project No. 20050732

(Fund 405734)

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September 2010

SUMMARY

The overall objective of this research was to modify the physicochemical and structural properties of bovine connective tissue, especially from mature animals, in order to improve the tenderness of tougher cuts and further processed products. Approaches include mechanical, chemical and thermal treatments alone and in combination.

During this project, work was carried out on several sub-objectives. Thermal and chemical properties of intramuscular connective tissue from cattle of two age groups (heifers – 17 months and cows – ~ 6 years) were studied and compared with the shear force data of meat from same muscles to understand the relationship. Also, the combined effect of heating time and temperature in modifying epimysial toughness of mature cows was studied. The treatment combinations included low (55°C), medium high (70°C) and high temperatures (80 and 95°C) and varying lengths of time, for example, a wide time range at low temperature (15 min to 24 h), medi m time range (5min to 3 h) at medium high temperature and short time range (5 min to 1 h) at high temperature. Further, studies were conducted to assess the effectiveness of combined treatments of moisture enhancement, blade tenderization and heating regime (moist and dry heat cooking) to improve tenderness of steaks and roasts from mature cattle. The effect of several concentrations of two alkalis (sodium and ammonium hydroxide) on epimysium has been investigated with the combined treatments of pre-equilibration in alkali and heating at 55 and 70°C. Another study was conducted on the sensory properties of meat logs made from restructured beef from animals of two age groups to determine the effects of flaking technology on meat properties.

During the work described above, a great deal was learned about the properties of collagen and connective tissue from young and mature beef cuts. The study of collagen chemistry and structure was especially challenging as this protein has unique biochemical properties which required different approaches than for myofibrillar proteins. However, many meat cuts from mature cattle may be quite acceptable to consumers as value-added products, given suitable postmortem tenderization strategies which specifically target the connective tissue.

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BACKGROUND

A consistent product with a high degree of value for the consumer and a reasonable cost of production for the producer, processor and retailer are the main focus of the meat industry. The value of the finished product depends on various quality attributes, namely taste, consistency, tenderness, healthfulness, convenience and perception. Consumer satisfaction guarantees repeat purchase of the product and new market opportunities. Studies conducted over the last century related meat tenderness and toughness to properties of myofibrillar and connective tissue proteins. Connective tissue proteins are dominated by collagen which is partly soluble and the insoluble portion contributes significantly to meat toughness.

Objectives

The overall objective of this study is to modify the physicochemical and structural properties of bovine connective tissue in order to improve the tenderness of tougher cuts and further processed products from mature beef. Approaches include mechanical, thermal and chemical treatments alone and/or in combination.

Sub-objectives:

1) Understand the chemical composition of connective tissue (especially that of collagen) in terms of quantity, distribution, type) and differences due to animal maturity and to develop methodologies to influence properties of the connective tissue.

2) Investigate various thermal treatments to improve solubilization of collagen to increase tenderness.

3) Evaluate the efficacy of chemical and mechanical treatments on properties of connective tissue/meat cuts from mature animals.

4) Investigate process methods for chopped and formed beef rolls.

Activity 1: The effects of age of animal and type of muscle on the thermal properties of meat proteins, mainly connective tissue proteins, and toughness/tenderness attributes.

1.1 Introduction

Intramuscular connective tissues are located within a muscle and have long been considered a major contributor to the toughness of beef. Its composition, amounts and morphology vary from muscle to muscle within a species (Light et al., 1985; Jarvinen et al., 2002 and Rhee et al., 2004). The connective tissue network of a muscle is organized in three hierarchical domains defined as epimysium- intermuscular connective tissues, perimysium and endomysium- intramuscular connective tissues. The protein collagen is the main constituent of all connective tissue. The epimysium and perimysium connective tissue were the main focus of this experiment.

These experiments were carried out to characterize selected bovine muscles and to evaluate effect of the age of the animal and type of muscle on the thermal properties of meat proteins, mainly connective tissue proteins, and toughness / tenderness attributes.

1.2. Materials and methods

1.2.1 Meat sample preparation

Representing two maturity groups, six cows of Canada grade D2 (6 ±1 years old; carcass weight of 331±64 kg) and six heifers of Canada grade Y1 (16±2 months old; carcass weight of 287±31 kg) were used in this study. Animals were slaughtered at a provincially inspected abattoir and carcasses After 48 h b*iceps femoris* (BF), *gluteus medius* (GM), *longissimus* (L) *and semimembranosus* (SM) muscles were excised. Meat samples were prepared on the 14^{th} day post-mortem. Each muscle, except GM, was divided into three portions; anterior, posterior and middle. GM was divided into a dorsal and two other portions along fibre directions. From each portion ~2.5 cm thick steaks were prepared after saving a slice for chemical analysis. All samples were vacuum-packed and stored at -30°C until used.

1.2.2 Isolation of intramuscular connective tissue (IMCT)

Perimysium and endomysium termed IMCT were excised from muscles using Fujii and Murota (1982) procedure. Briefly, 200 g of ground meat was mixed with 1 L of 10 mM Tris-maleate/0.1 M KCl at pH 7.2 and homogenized. The homogenate was stirred for 12 h/4°C. Insoluble material was collected and stirred for 12 h/4°C x 3 with 1 L of Hasselbach-Schneider solution (0.6 M KCl, 10 mM $Na_4P_2O_7$, 1 mM $MgCl_2$ and 0.1 M KH_2PO_4 at pH 6.4) each time. Insoluble was stirred for 12 h/4°C x 2 with 0.5 L of 0.6 M KI/0.06 M $Na_2S_2O_3$ and then washed with water. The insoluble material collected was IMCT.

1.2.3 Meat and IMCT protein denaturartion temperatures

About 10 mg of ground meat /IMCT was weighed into aluminum pans and hermetically sealed. The pans were heated from 20°C to 100°C at 3°C/min using a differential scanning calorimeter (DSC 2010, TA Instruments, Delaware, USA).

1.2.4 Total collagen determination

Total collagen was determined according to the AOAC 990.26 (1990) procedure. About 4.0 g of ground meat or 0.4 g of oven dried IMCT/epimysium was hydrolyzed overnight in 3.5 M H_2SO_4 at 105 ± 1°C. The digest was diluted with de-ionized water as required and then filtered. An aliquot of 2 mL was mixed with 1 mL of 1.41% chloramine T (w/v) and incubated for 20 min at room temperature. It was mixed with 1 mL of colour reagent (10 g 4-dimethylaminobenzaldehyde, 35 mL of 60% perchloric acid (w/w) and 65 mL of 2-propanol) and heated for 15 min/60 ± 1°C. The absorbance of cooled samples was measured at 558 nm using a UV-VIS spectrophotometer (Genesys 5, Milton & Roy Spectronic, Ivyland, PA, USA). Calibration curves were prepared using trans-4-hydroxy-L-proline. Hydroxyproline was converted to collagen by multiplying with a factor of 8.

1.2.5 Ringer's soluble and thermolabile collagen determination

Ringer's insoluble collagen of meat and water soluble collagen of epimysium after heat treatments were analyzed for collagen as per AOAC 990.26 (1990) procedure. Ringer's soluble collagen (Hill, 1966) of meat was computed as the difference between total collagen and Ringer's insoluble collagen. About 6 g of ground meat mixed with 12 mL of ¼ strength Ringer's solution in 30 mL polypropylene tubes and heated for 1 h/77±1 °C in a pre-heated water bath. After cooling, samples centrifuged at 2800 x *g* (Beckman JC-HC, Beckman Instruments, Palo Alto, CA, USA) for 30 min/20°C. The pellet mixed with 8 mL of ¼ strength Ringer's solution and then centrifuged. The pellet was used to determine insoluble collagen.

1.3. Results

1.3.1 Meat and IMCT protein denaturartion temperatures

Meat protein denaturation started at ~46.5°C and ended at ~77.9°C and that was consistent across muscles. DSC thermograms showed three endothermic peaks for meat proteins at 52, 62 and 72°C and were designated as peak I, II and III, respectively. As previously reported, these peaks represented denaturation of myosin, collagen and actin at 40-60 °C, 56-62 °C and 66-73 °C, respectively (Martens et al., 1982). The temperatures for peaks I and III were constant across muscles and also between cows and heifers (Table 1). Peak II temperatures were influenced by the maturity*muscle interaction ($P<0.05$). That is peak II temperatures of BF and L muscles from cows were significantly higher than that from heifers but peak II temperatures for SM and GM were not different between cows and heifers. The muscle (type) and animal maturity had no effect on the energy used for protein denaturation (J/g of raw meat). Denaturation of IMCT

proteins started at ~51.1-55.8 °C and ended at ~67.2-70.9 °C with a single peak at 59.2 - 62.7 °C (Table 1). The peak temperature of IMCT from GM significantly varied from that of other muscles (P<0.001). However, the values were similar between cows and heifers for any given muscle.

Table 1.1 Thermal properties of meat and intra muscular connective tissue proteins

		Thermal denaturation temperature (C°)			
		Peak I	Peak II	Peak III	Peak IMCT
Maturity	Cow	52.6 ± 0.2	72.5 ±0.4	61.3 ± 0.9	61.3 ± 0.9
	Heifer	52.6 ± 0.4	72.4 ± 0.4	61.0 ± 0.8	61.0 ± 0.8
	P	0.925	0.783	0.688	0.688
	LSD	-	-	-	-
Muscle	SM	52.5 ± 0.2	72.3 ± 0.4	62.7 ± 0.5	62.7 ± 0.5
	BF	52.6 ± 0.2	72.5 ± 0.4	61.3 ± 0.8	61.3 ± 0.8
	GM	52.7 ± 0.2	72.5 ± 0.3	59.2 ± 0.8	59.2 ± 0.8
	L	52.7 ± 0.5	72.7 ± 0.3	61.3 ± 0.8	61.3 ± 0.8
	P	0.853	0.605	0.0004	0.0004
	LSD	-	0.64	1.4	1.4

Means ± SE are presented (P<0.05).
BF - *biceps femoris.* GM - *gluteus medius. L* - longissimus. *SM* - semimembranosus.

1.3.2 IMCT and total collagen

The quantity of IMCT is <10% of the dry weight of meat. IMCT content varied between heifers (5.8 g/100 g, db) and cows (7.4 g/100g) (table 2). Among muscles investigated GM meat contained the highest levels of IMCT (~9.4 g/100 g, db) and followed by BF meat (7.8 g/100 g, db). Meat of SM and L contained similar levels of IMCT, ~5.1 and 4.2 g/ 100 g (db), respectively. Observed muscle related differences in IMCT contents might be related to the functional needs of skeletal muscles.

Total collagen content of isolated IMCT was similar for heifers (41.1 g/100g) and cows (41.3 g/100g) and among muscles studied (Table 2). This is a significant observation that is collagen g/100 g IMCT (db) is a constant regardless of animal maturity and muscle variability. However collagen on a meat basis showed that cow meat (0.85 g/100g) contained high amounts of total collagen than heifer meat (0.69 g/100g) and total collagen content varied across muscles (Table 2). This may be related to the composition variability observed between various muscles and maturity groups (Table 3). However, total collagen content of GM meat might not be indicative of that of a GM steak as the distinct band of connective tissue in GM is usually removed during portioning. Collagen had contributed to 5.8, 4.6, 2.8 and 2.7% of proteins *[(Total collagen g/Total*

*protein g)*100]* of cow meat from GM, BF, SM and L, respectively. Collagen had contributed to 4.6, 3.6, 2.4 and 2.2% of proteins in GM, BF, SM and L from heifers.

Table 1.2 Total and soluble collagen of meat and IMCT

		IMCT (g/100g meat, db)	Total collagen (g/100g meat, wb)	Total collagen (g/ 100g IMCT, wb)
Maturity	Cow	7.4 ± 1.5	0.85 ± 0.04	41.3 ± 2.9
	Heifer	5.8 ± 0.8	0.69 ± 0.04	41.1 ± 3.5
	P	0.027	0.0.042	0.842
	LSD	1.092	0.083	-
Muscle	SM	5.1 ± 0.5	0.57 ± 0.04	38.1 ± 3.1
	BF	7.8 ± 0.5	0.86 ± 0.10	41.4 ± 3.0
	GM	9.4 ± 1.5	1.11 ± 0.10	43.6 ± 3.8
	L	4.2 ± 0.4	0.54 ± 0.04	41.7 ± 2.7
	P	0.0001	0.0001	0.505
	LSD	1.54	0.12	-

Means ± SE are presented (P<0.05).
BF - *biceps femoris*. GM - *gluteus medius*. *L* - longissimus. *SM* - semimembranosus.
db - dry basis, wb - wet basis

Table 1.3 Percent contents of protein and moisture and pH of meat

	Heifer				Cow					
	BF	SM	GM	L	BF	SM	GM	L	P	LSD
Protein	21.1 ± 0.3	22.2 ± 0.2	21.4 ± 0.8	21.8 ± 0.2	21.1 ± 0.2	21.8 ± 0.2	21.2 ± 0.2	21.7 ± 0.3	0.007	0.6
Moisture	73.2 ± 0.4	74.2 ± 0.1	72.9 ± 0.3	72.0 ± 0.3	74.6 ± 0.4	74.8 ± 0.4	73.4 ± 0.5	73.4 ± 0.4	0.0001	1.1
pH	5.4 ± 0.04	5.4 ± 0.1	5.3 ± 0.04	5.4 ± 0.1	5.3 ± 0.04	5.3 ± 0.04	5.4 ± 0.04	5.3 ± 0.04	0.473	-

Means ± SE are presented. N=6.
BF-*biceps femoris*; SM-*semimembranosus*, GM-*gluteus medius*, L-*longissimus*.

1.3.3 Soluble collagen

At 77°C, collagen solubility in Ringer's solution was <0.5% on a meat basis for all muscles investigated (Figure 1a). Presented on total collagen basis, collagen from cow meat was less soluble than that from heifers, except for meat from BF for which collagen solubility was similar between cows and heifers (Figure 1 b). GM from heifers showed the highest solubility of collagen but would not represent that of a steak

because the visible band of connective tissue was removed before cooking. Collagen from cow GM and L meat was three times less soluble than that of heifer meat (Figure 1b). Collagen from SM meat from cows was twice as less soluble as collagen from heifers. This indicates maturity/age related changes influence collagen solubility only in certain muscles but in others, i.e. BF, functional needs surpass maturity.

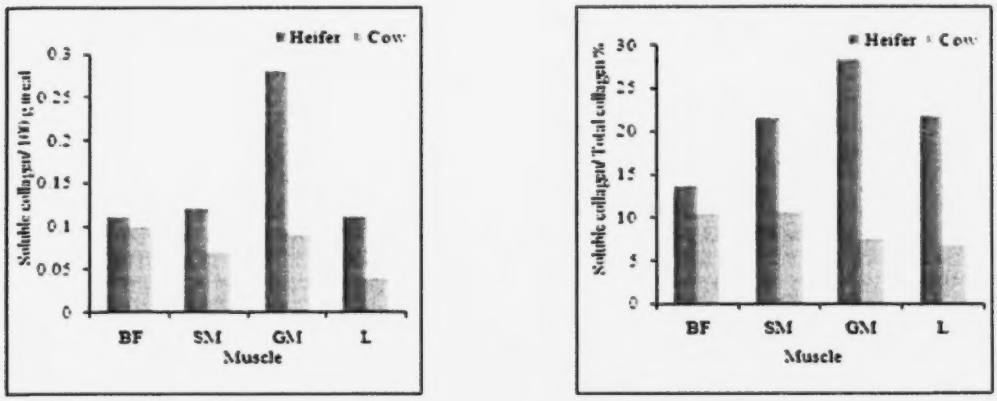


Figure 1.1 Collagen solubility presented on a (a) meat basis and (b) total collagen basis.

1.3.4 Shear force

The highest force required for shearing a meat sample was reported as the maximum shear force or SF (max). Myofibril driven shear force or SF (myo) was the first peak and the connective tissue driven shear force or SF (con) was the second peak to appear on force deformation curves. The SF (max) can be originating from either connective tissue or myofibrillar proteins. The SF (max) and SF (con) were significantly influenced by the interaction between animal maturity and muscle (Figure 2). Myofibrils contributed to the SF (max) values of GM and L meat but connective tissue had contributed to the SF (max) values of BF meat. However, myofibrils contributed to the SF (max) values of SM meat from heifers but that of cows was contributed equally by myofibrils and connective tissues. Out of all muscles studied, the highest values for SF (con) were observed for BF meat from cows and the lowest values were reported for L meat from heifers. Similar SF (con) values were reported for GM and L meat from cows. The SF (max) values of cow meat were higher than that of heifer meat except for GM, for which values were similar for cows and heifers. However, it was noted that the reported shear force values for GM muscles could be lower than the actual because the thick band of connective tissue in GM muscle was eliminated from samples to attain structural uniformity.

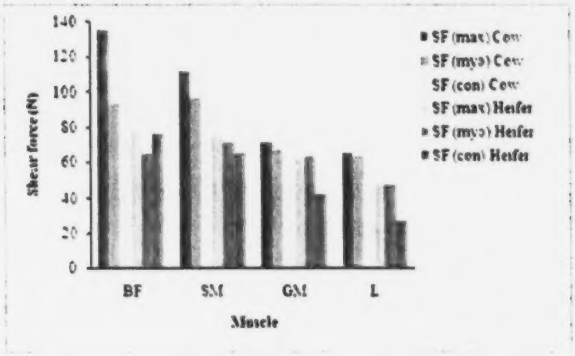


Figure 1.2 Shear force values of meat from different muscles. BF – *Biceps femoris*. SM – *Semimebranosus*. GM – *Gluteus medius*. L – *Longissimus*.

1.4 Conclusions

This study confirms that the muscle (type) and maturity/age of animals determines the amount of intramuscular connective tissue in meat from BF, SM, GM and L. The ratio of collagen to IMCT is a constant for muscles studied irrespective of animal; maturity and therefore total collagen is a good indicator of connective tissue content of muscles. The decreasing solubility of collagen with increasing animal maturity is not a universal phenomenon and collagen solubility can be governed by factors other than animal maturity. Based on thermal denaturation temperatures, IMCT proteins, mainly collagen, denatures at 51.1-70.9°C during heating. The quantity of IMCT does not predict the shear force values of BF, SM, GM and L meat.

Personnel Involved

A. Perera (Ph.D. student), Z. Pietrasik, P.J. Shand, H. Silcox and N.Wiebe

Funding for this activity

Some of the materials for this study were provided with funds from the Canadian Cattlemen's Association. Also, part of Dr. Pietrasik's salary was provided by the Value-Added Beef Program (funded by the Saskatchewan Beef Development Fund).

Communication of results

Perera*, K.A.A., Pietrasik, Z. and **Shand, P.J.** 2007. Effects of heating time and temperature on shear force and physiochemical characteristics of bovine *longissimus* epimysium. 60th Annual Reciprocal Meat Conference of the American Meat Science Association, June 17-20, Brookings, SD. Abstract # 79 [oral KP] *graduate student.

Activity 2: Effects of cooking regimes on modifying the toughness of beef connective tissue

2.1 Introduction

Cooking has a major influence on the tenderness of a meat product as the water- and fat-binding characteristics, as well as the texture, are closely related to the conditions of heating. Selecting an appropriate heating rate is important to determine the quality of final product, and is particularly important for lesser value cuts with high levels of connective tissue.

Myofibrillar and connective tissue proteins (mainly collagen) are responsible for major textural changes in meat during heating (refer to activity 1). It is accepted that heat solubilises connective tissue collagen and that results in meat tenderization whereas heat denatures myofibrillar proteins and that results in meat toughening. These heat-induced changes are time and temperature dependent.

Myofibrillar proteins exposed to thermal energy (heating) are denatured and that results in protein coagulation and loss of solubility. Collagen of the connective tissue undergoes physical changes during heating, i.e. swells, in the presence of moisture, and shrinks. With extended heating in the presence of moisture, collagen is further hydrated and hydrolyzed, to make meat tender. During cooling, soluble collagen forms a gel network. As such, heat causes some tenderization and toughening of meat simultaneously due to its variable effect on different constituents of meat proteins.

Changes that take place in connective tissue embedded in the muscle matrix were previously studied. Since the triple helix collagen molecule is stabilized mainly through hydrogen bonds it is hypothesized that under conditions favourable for dissociation of hydrogen bonds the strength of the collagen dominant connective tissue is reduced but this tenderness improvement is not observed (is masked) in the muscle matrix under similar conditions. Studying the thermal behaviour of connective tissue per se was considered a useful exercise to understand the true nature of changes that take place in connective tissue in the muscle matrix. In order to obtain sufficient material of uniform thickness to work with, a decision was made to replace perimysium (as originally planned) with epimysium.

2.2 Materials and methods

2.2.1 Epimysium sample preparation and treatments

Locally purchased bovine *longissimus* muscles from 4 heifers (17 months) and 4 cows (60-88 months) were used in this experiment. The dorsal epimysium with a layer of meat was excised on the 7th day post-mortem and stored at –20°C. After removing fat and muscle fibres, two adjacent strips of epimysium were cut along the length to prepare 2 x 2½ cm pieces (1.4 ± 0.4 g) and those cut from a single strip were treated at the same temperature. Epimysium pieces from right side muscles were later heated at 55 and 70 ± 2°C and those from

the left side were treated at 80 and 95 ± 2°C with 10 mL of de-ionized water. Heating times employed were as follows: at 55°C 15, 30, 45, 60, 180, 540 and 1440 min; at 70°C 5, 10, 15, 45, 60, 180 and 360 min, and at 80 and 95°C 5, 10, 15, 45 and 60 min. Two raw epimysium pieces (2 x 2½ cm) cut from each strip were stored at -20°C to determine shear stress later. Treatment subsamples were prepared from the adjacent strips. The remaining epimysium was ground and stored at -20°C for chemical analysis.

2.2.2 Properties of epimysium

The frozen epimysium was diced and then repeatedly ground using a Waring commercial blender (Waring Products, Torrington, USA) with alternate freezing in liquid N_2 to obtain fine fibres. Moisture (AOAC 950.46 B, 1990), crude protein (AOAC 981.10, 1990), crude fat (AOAC 960.39, 1990) and collagen (AOAC 990.26, 1990) contents of ground epimysium were determined in duplicate to characterize the raw epimysium.

2.2.3 Determination of shear force (SF)

Epimysium samples, both heat treated and raw, pre-cooled to 4° C were cut into 3 strips ½ cm wide, along the direction of the fibres (visible lines on the cleaned epimysium). Strips at room temperature were sheared perpendicular to the fibre direction using TMS-Pro texture system (Food Technology Corp., Sterling, VA, USA) equipped with a Warner-Bratzler shear attachment. Maximum force generated during shearing was recorded as shear force and corrected for the thickness of raw epimysium.

2.2.4 Hydrolysis with Pronase

Previous work carried out by Snowden et al. (1978) and Powell et al. (2000) demonstrated that enzymatic digestion of heat treated collagen (free from soluble collagen) with pronase release denatured but insoluble portion of collagen fibres, refer to as amorphous collagen. Based on this observation the following analysis was carried out. After measuring SS, epimysium strips were finely minced. About 0.8g was then weighed into 10.4 mL Beckman™ polycarbonate centrifuge bottles (Beckman Instruments, USA). Then it was mixed with 9 mL of 0.5mg/mL pronase solution prepared in 0.12M Tris / 0.1M $CaCl_2$ buffer (Scott et al. 1981). Samples were placed on a shaker and hydrolysis was carried out for ~16 h at room temperature. At the end, samples were subjected to ultracentrifugation using a Beckman Coulter Optima™ centrifuge with a 70.1Ti rotor and at 80 000 x g/20°C. Clear supernatant was collected and the volume was adjusted to 10 mL with de-ionized water before storage at -20°C. The pellet was discarded. Total protein and Ehrlich chromogen (cross-link) contents of the supernatant were determined.

2.2.5 Determination of total protein – BCA protein assay

Hydroxyproline would be the most appropriate indicator of collagen in connective tissue. However, previous work revealed that AOAC 990.26 (1990) colourimetric assay was not sensitive to detect very low levels of hydroxyproline. Therefore, BCA protein assay (Smith et al., 1985) was used to measure protein liberated

from epimysium during heating in water, refer to as thermolabile protein (TLP), and protein in the pronase digest refer to as amorphous protein (AMP) as indicators of denatured epimysial proteins

The BCA™ protein assay reagent A (containing sodium carbonate, sodium bicarbonate, sodium tartrate and BCA detection reagent in 0.1N sodium hydroxide) and BCA™ protein assay reagent B (4% cupric sulphate pentahydrate) were mixed in a 100:2 mL ratio, respectively. Thermolabile proteins in epimysium extracts were diluted appropriately before mixing with assay reagents. In a test tube, 2 mL of the assay reagent mix (already prepared) was added to 100 µL of the diluted sample. Contents were immediately mixed on a vortex and then heated at 60°C in a water bath for 30 minutes. Contents were cooled to room temperature before measuring absorbance at 562 nm using UV-VIS spectrophotometer (Genesys 5, Milton & Roy Company, USA). Protein standards were prepared using type B gelatin from bovine skin (Sigma, Canada).

2.2.6 SDS-gel electrophoresis

Gel-electrophoresis was carried out to understand the peptide profile of thermolabile proteins. Epimysium extracts preserved at -20°C was thawed to room temperature. Based on protein data from the BCA assay, volumes required to obtain 50 µg of dry protein were calculated. Then exact volumes from the selected treatments were lyophilized in 1 mL micro centrifuge tubes. Then the whole lyophilized sample was mixed with 100 µL of twice concentrated sample buffer (0.1 M Tris at pH 6.8, 2% sodium dodecyl sulphate, 20% glycerol, 0.001% bromophenol blue and 38% de-ionized water) and 2-mercaptoethanol mixed in 19:1 ratio (v/v), respectively. The mixture was heated for 5 min at 95°C. Resolution was carried out on polyacrylamide gels of 8% resolution and 4% stacking phases. Each well of a gel was loaded with 3 µg of protein. Electrophoresis was carried out at 176 V, in Tris (3.025 g/L) and glycine (14.415 g/L) buffer at pH 8.2. Gels were stained (0.01% coomasie brilliant blue, 50% methanol and 7% acetic acid) for 5 min and then de-stained (10% acetic acid, 7.5% methanol) overnight. Molecular weight marker was purchased from (Bio-Rad Laboratories, Hercules, CA, USA.

2.2.7 Determination of Ehrlich chromogen (EC) content

Some of the cross-links of collagen must have broken to liberate α (single) and β (double) chains (as shown on SDS-gels). As such it was considered a useful exercise to determine the ehrlich chromogen (Scott et al., 1981) content in the supernatants from the pronase extractions as enzyme action on (heat-induced) amorphous collagen may also release some cross-linked molecules. The reagents used were 5% dimethyl amino benzaldehyde in 4N perchloric acid/0.01% mercuric chloride. Samples and reagents were mixed in 5.25:1 (v/v) ratio in 2 mL micro tubes. The mixture was centrifuged immediately for 30 s at 12 000 rpm using a 5415 C Eppendorf Centrifuge (Brinkmann Instruments Inc, USA) and at room temperature. Four minutes after mixing the sample and reagent, absorbance was measured at 572 nm using UV-VIS spectrophotometer (Genesys 5, Milton & Roy Company, USA).

2.3 Results and discussion

The connective tissue sheath around the *longissimus* muscle, regardless of its thickness or macroscopic appearance was considered as epimysium in this study.

Table 1 shows the basic properties of epimysium. Epimysium from heifers had ~38% protein on a fresh weight basis and collagen contributed to ~88.5% of that. Epimysium from cows had ~43.5% protein and ~91% of that was collagen. Raw cow epimysium (~38.6 N/mm^2) showed higher SS values than heifer epimysium (~ 32 N/mm^2).

Table 2.1 Characterization of epimysium (Mean ± SE, n=4)

	Heifer	Cow	P
Protein (g/100g wb)	38.4 ± 0.8	43.5 ± 2.2	0.02
Fat (g/100g wb)	16.4 ± 1.3	9.3 ± 2.0	0.03
Moisture (g/100g wb)	47.2 ± 0.9	47.4 ± 1.7	0.83
Collagen (g/100g wb)	36.4 ± 1.4	40.1 ± 2.1	0.11
Collagen (g/100 g Protein)	88.5 ± 4.0	91.4 ± 4.5	0.01
Shear force(N/mm^2)	32.0 ± 1.6	38.6 ± 3.9	0.02

wb – wet basis

2.3.1 Shear Force (SF) data

The changes in SF with the heating time at 55, 70, 80 and 95°C are graphically presented in Fig 1 (a),(b), (c) and (d), respectively. Interestingly, SF values of mature epimysium were higher than that of heifer epimysium at all temperatures investigated and across all heating times employed. For instance, at the end of 24 h heat treatment at 55 °C, SF values of heifer epimysium were dropped to ~9 N while that of cow epimysium was dropped to only 104 N. SF values were decreased faster (at shorter heating times) at 70°C than at 55°C. As temperature was increased to 80 and 95°C, SF values were decreased further for both cow and heifer epimysium.

2.3.2 Thermolabile (TLP) and amorphous proteins (AMP)

Thermolabile protein release from the epimysium was significantly influenced by the interaction between maturity*heating time at both 55 ($P<0.05$) and at 70°C ($P<0.05$) and which is graphically presented in Figure 2.2 (A-H). At each temperature investigated long heating times increased the amounts of TLP released. However, the amount of TLP released by heifer epimysium after 1440 min heating at 55°C and 360 min heating at 70°C were significantly higher than TLP released at all other treatments. These amounts were several folds higher than that released by cow epimysium under similar conditions of heating. This predicts

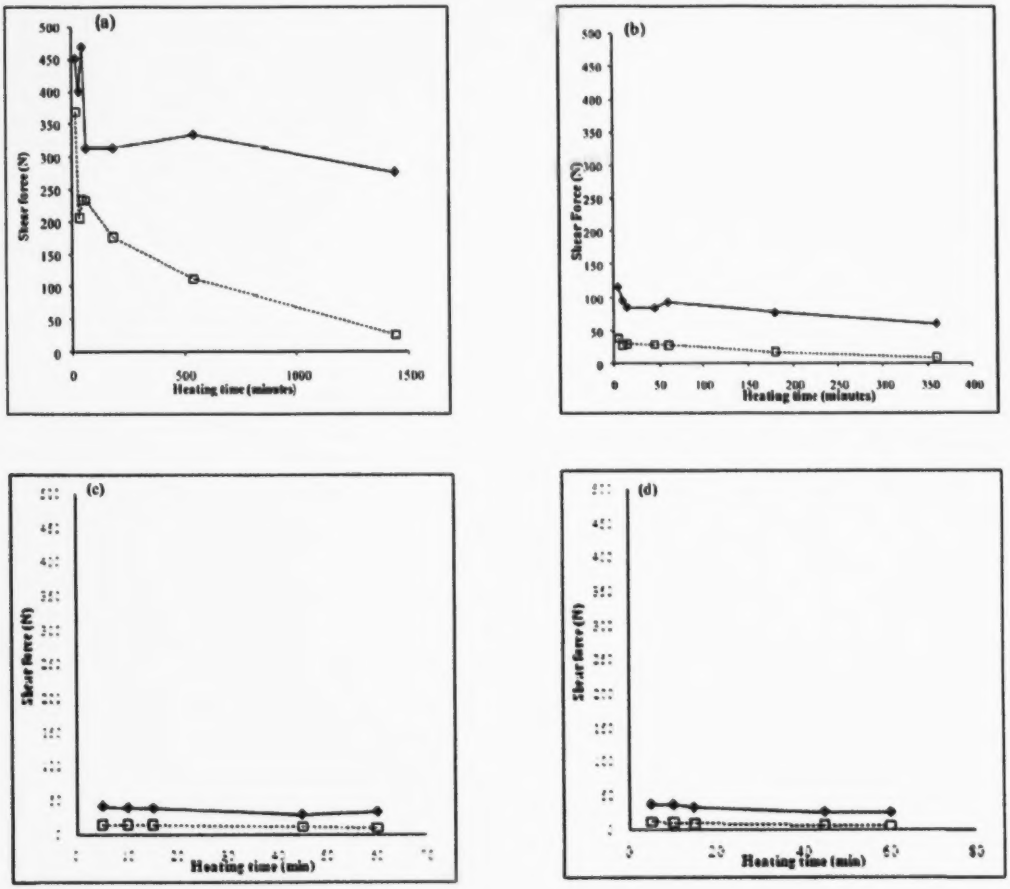


Figure 2.1 Changes in epimysial shear force with heating time. (a) 55°C. (b) 70°C. (c) 80°C. (d) 95°C. Open symbols represent data of heifer epimysium and closed symbols represent data of cow epimysium.

the thermal resistance of cow epimysial proteins. Also, this indicates that long durations of heating at moderate temperatures can solubilise more collagen from heifer epimysium. Maturity of animals had shown a tendency to have a significant effect on the release of TLP at 55 and 70°C (P=0.05), that is heifer epimysium released more TLP than cow epimysium (Figure 2.2, A-D). During 60 min heating time, the amounts of TLP released from epimysium were gradually increased with increasing temperature. The amounts of AMP produced were considerably low at 55°C for both cow and heifer epimysium. AMP levels reached the highest values reported just after 5 min heating at 70°C and the slight increase in AMP with heating time was not statistically significant. Heating epimysium at temperatures beyond 70°C did not increase the amounts of AMP formed. There was a tendency to decrease AMP with the long heating times (360 min) at 70°C. When AMP was maintained at constant levels at various heating times and at ≤ 70°C, shear force values were continued to decline for both cow and heifer epimysium. Thus, AMP levels should not have an effect on the decreasing shear force values of epimysium.

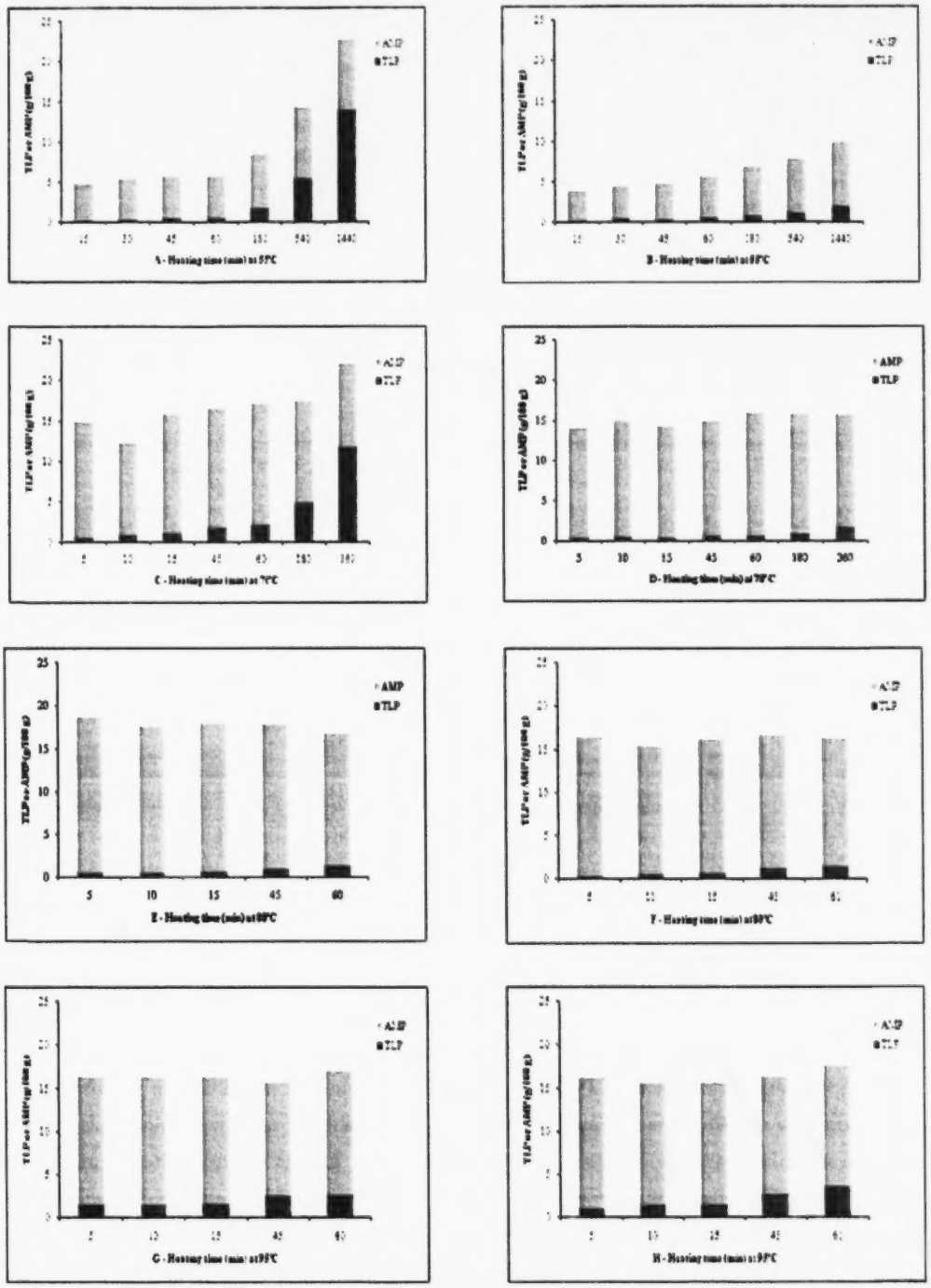


Figure 2.2 Changes in the amounts of TLP and AMP of epimysium in relation to temperature and heating time. A – Heifer epimysium at 55°C. B – Cow epimysium at 55°C. C - Heifer epimysium at 70°C. D – Cow epimysium at 70°C. E – Heifer epimysium at 80°C. F – Cow epimysium at 80°C. G – Heifer epimysium at 95°C. H – Cow epimysium at 95°C.

2.3.3 SDS-gel electrophoresis

At 55 °C, TLP of heifer (Figure 2.3a) and cow (Figure 2.3b) epimysium contained single strands (α1 and α2 chains, >116 kDa) and double strands (2α chains, >200 kDa) of collagen with other unidentified connective tissue proteins. As previously reported, the molecular weight of an α chain of collagen is about 100kDa and that o 2α chains is about 200kDa (Gómez-Guillén et al, 2002; Dreisewerd et al., 2004). Heifer epimysium released single and double strands of collagen starting from 15 min heating at 55 °C but cow epimysium started releasing them only after 30 min heating at 55 °C. Based on the band thickness, heifer epimysium released more double strands than cow epimysium at corresponding heating times. Molecular weights of protein bands produced at 70 °C were similar to those observed at 55 °C for both cow and heifer epimysium, however band thickness increased at 70 °C indicating liberation of more proteins than at 55 °C (gels not shown).

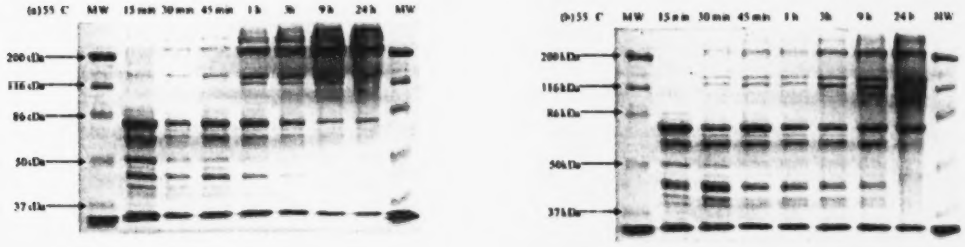


Figure 2.3 – SDS-PAGE showing peptides released during heating of epimysium at 55°C. Molecular weight markers (MW) are placed at the two ends of the gels. (a) Peptide profile of TLP from heifer epimysium at 55°C. (b) Peptide profile of TLP from cow epimysium at 55°C. Peptide bands at 116 and 200kDa are single (α1 and α2) and double (β) chains of collagen.

2.3.4 EC cross-links

EC (pyrrolic) cross-links in AMP were measured as indicators of thermal damage to epimysium. EC contents were reported as absorbance at 572 nm per unit weight (g) of AMP due to non-availability of EC standards. EC released with the amorphous collagen was neither influenced by the maturity of animals nor by the heating times at both 55 and 70 °C (Figure 2.3). Apparently, temperature increase from 55 to 70 °C also had no effect on the amount of 'EC/AMP' released from cow and heifer epimysium. As the amount of EC present in a unit weight (g) of AMP was observed to be a constant it was assumed that EC cross-links were uniformly distributed in amorphous areas of collagen. A preliminary study carried out on EC contents of thermolabile protein extracts from epimysial heating at 55 and 70 °C had shown consistent but almost negligible absorbance values for all treatment combinations. Therefore, analysis of EC in thermolabile protein extracts was abandoned.

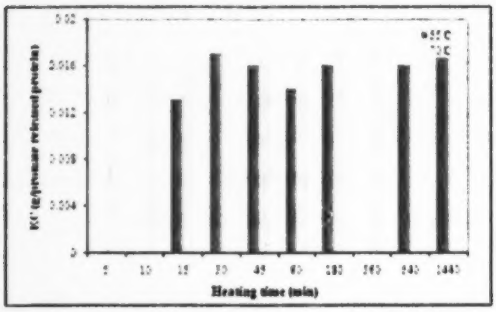


Figure 2.3 Changes in EC content of AMP during heating at 55 and 70°C.

2.4 Conclusions

Collagen contributes to about 90% of epimysial proteins. Physicochemical properties of epimysium should, therefore, be governed by the properties of collagen. Epimysial properties assessed, except for shear stress, did not change with the animal maturity, heifers vs. cow. The conversion of collagen from a native state to an amorphous state (extracted by pronase) was not related to animal maturity and changes in shear force. Shear force values of heifer epimysium can be significantly decreased by heating at 70°C and as heating time increased. Shear force values of cow epimysium can be decreased by increasing both temperature (>70°C) and heating time.

Personnel Involved

A.Perera (Ph.D. student), Z. Pietrasik, P.J. Shand, H. Silcox, N.Wiebe and Derrick Pobereznek

References

References will be provided on request

Communication of results:

'Effects of heating time and temperature on shear force and physicochemical characteristics of bovine *longissimus* epimysium' *by* Anula Perera, Zeb Pietrasik, Phyllis Shand. An oral presentation at the Reciprocal Meat Conference, June 17-20, 2007, South Dakota State University, Brookings, South Dakota.

A manuscript is ready to be submitted to a peer reviewed journal.

Funding for this activity

Some of the materials for this study were provided with funds from the Canadian Cattlemen's Association. Also, part of Dr. Pietrasik's salary was provided by the Value-Added Beef Program (funded by the Saskatchewan Beef Development Fund).

Activity 3: Evaluate the efficacy of mechanical treatments on properties of connective tissue/meat cuts from mature animals

3.1 Introduction

Conditions of cooking have a major influence on the tenderness, water- and fat-binding characteristics, as well as overall texture of meat. Selecting an appropriate cooking regime is important in determining the quality of a meat product, and is particularly important for lesser value cuts with high connective tissue content.The objective of this study was to determine the combined effect of moisture enhancement, blade tenderization and different cooking regimes on the cooking yield and tenderness of selected beef muscles from youthful and mature animals.

3.2 Materials and methods

3.2.1 Sample preparation and treatment

Six beef heifers (AA or higher), and six D2 cows were slaughtered and dressed at a provincially inspected abattoir. Following 48 h chilling at 4°C, the *semimembranosus* (SM), *biceps femoris* (BF) and *longissimus* (L) muscles from both sides of carcasses were collected, coded for identity, and vacuum packaged. All muscles were aged at 3°C until 12th day of postmortem. On the day 12, muscles were trimmed of visible, external fat and connective tissue and cut into three sections, designated as anterior, central and posterior. Pooled samples of each muscle pair were collected to determine proximate composition, total collagen and pH. The roasts from one side were assigned to one of the two treatments, i.e., non-injected beef (C), or blade tenderized (BT). The roasts from the other side received one of the following treatments: moisture enhanced treatment (ME) and blade tenderized followed by moisture enhancement (BT+ME). The 'C' roasts were placed directly into bags, vacuum packaged and refrigerated for 48 h at 4°C. The BT and BT+ME roasts were treated as planned by passing once through a Jaccard®, Model H # B4590 meat tenderizer (Jaccard®, Orchard Park, NY, USA).

After blade tenderization, BT roasts were vacuum packaged. ME and BT+ME roasts were injected using a Reiser Fomaco multi-needle injector (Model FGM 20/40, Fomaco Reiser Ltd., Burlington, ON) with brine to give 0.4% salt and 0.3% sodium tripolyphosphate in the final product. After injection roast weight reached 112% over the raw meat weight. The roasts were weighed before and after injection to determine the percent brine pick up (i.e. injected weight/raw weight x 100). Those roasts which did not reach their target weight were further injected with the marinade using a hand injector. After injection, the muscle samples were vacuum packaged and stored for 48 h at 4°C.

At approximately 48 h post-injection, all samples were removed from packaging and weighed to determine the retained injection level or drip loss ({wt at 48 h/raw wt} x 100). Then, two 2.54 cm thick steaks were cut perpendicular to the muscle fiber orientation. The steaks were vacuum packaged, frozen in a blast-freezer and

stored at –30°C until used. These steaks were used for the evaluation of tenderness after dry- and moist-heat cooking (*i.e.*, cooked to an internal temperature of 72°C). At the completion of appropriate storage period, frozen samples were thawed (4°C for 24 h), steaks prepared and then cooked similarly to aged samples for tenderness evaluation.

3.2.2 pH determination

The pH values of raw beef were measured in duplicate with a Fisher Accumet 915 pH Meter (Fisher Scientific, Nepean, ON, Canada) using a stab pH electrode.

3.2.3 Purge during storage

Purge was determined by reweighing steaks after removal from the main packaging following the 48 h storage interval, and expressed as a percentage of the initial steak weight.

3.2.4 Cooking of steaks

Steaks were thawed in a cooler at 4°C for 24 h and cooked using following methods:

a) Dry-heat cooking

Dry-heat cooking was carried out using an electric grill (Garland ED-30B electric grill; Condon Barr Food Equipment Ltd, Edmonton, AB). Steaks were placed on the grill, preheated to 210°C, cooked until the internal temperature reached 35°C, turned and cooked to 71°C.

b) Moist-heat cooking

Steaks assigned to moist-heat cooking were re-packaged into Cry-O-Vac CN 510 cook bags, vacuum packaged and then water cooked in an air-agitated water bath programmed to heat 50 min at 55°C, 40 min at 60°C, 30 min at 65°C and then maintain at 72°C. When the internal temperature reached 71°C sample bags were cooled down in a (tap) water bath until a temperature of 25°C was reached in the core. Cooking time / 100g = (the time taken by each steak to reach 71°C internal temperature / weight of the steak) x 100, was calculated for each steak. The temperatures of the steaks were monitored using the thermocouples attached to a scanner (Model 692-8000, Barnant Co., Barrington, IL, U.S.A.) and a computer, which recorded the change in temperature at 30 s intervals.

After cooling to an internal temperature of 25°C, cooked steaks were blotted dry with a paper towel and weighed to determine cook loss. Overall cook loss was calculated as the difference between the initial raw weight and weight after cooking, and expressed as a percentage of the original raw weight. Cooking loss = (raw weight at cooking -cooked weight /raw weight at cooking) x 100, was recorded for each steak.

3.2.5 Warner-Bratzler shear force (WBSF)

Shear force of 7-9 core samples (1.27x1.27x2.54cm), cut parallel to the fibre direction from each cooked steak, was determined. Samples were sheared perpendicular to the fibre direction using a TMS-Pro texture

system (Food Technology Corp., Sterling, VA, USA) fitted with a Warner-Bratzler shear attachment, and with crosshead speed set to 200 mm·min^{-1}. Force-deformation curves from the Warner-Bratzler shear device were used to determine WBSF myofibrillar component (SF-M) and WBSF connective tissue component (SF-C). The initial yield (height of the first peak) corresponded to SF-M and the final yield (height of the second peak) corresponded to SF-C, both of which were measured from the shear curves as suggested by Møller (1981). Maximum peak force recorded during the test was reported as WBSF.

3.2.6 Expressible moisture and hardness

Expressible moisture (EM) was determined on cooked steak samples by a modified method as described by Pietrasik & Li-Chan (2002). For each steak, EM was determined on three meat cubes (1.27 x 1.27 x 1.27cm) after equilibration to room temperature. Expressible fluid was measured gravimetrically after placing a pre-weighed meat sample (~2.5 g) between two layers of Whatman #3 filter paper, compressing the sample to 50% of original height with a 250 lb cell at a crosshead speed of 100 mm/min on a TMS-2000 texture system (Food Technology Corp., Sterling, VA, USA) and holding for 30 s. Results are expressed as the percentage ratio of moisture released from the sample to the initial beef sample weight. Additionally, force required to compress meat sample to 50% was reported as hardness.

3.2.7 Statistical analysis

The experimental design was a split-plot with six replications. Beef grades were the main plot treatment. Split-plots treatments included muscle type (*Biceps femoris, Semimembranosus* and *Longissimus*), four tenderization treatments (not treated control, blade tenderized, moisture enhanced, and blade tenderized followed by moisture enhancement) and cooking method (grilling, moist cooking). All variables were analyzed using SAS PROC MIXED. Grade, muscle, tenderization treatment, cooking method and their interactions were all considered to be fixed. Error terms specified in the model included replication (animal) x grade for the main plot; replication x grade x muscle for the first split; replication x grade x muscle x tenderization treatment for the second split; and residual error for the third split. Least-squares means were calculated for main effects or interactions that were represented by a significant F-test.

3.3 Results and discussions

3.3.1 Water holding capacity

There was no significant difference among grades in the amount of purge accumulated during 48 h storage period (Table 3.2). However D2 muscles tended to have ($P=0.07$) lower thaw drip compared to Y1 muscles. Brine retention of the muscles 48 h post-enhancement indicated that the SM and L muscles retained less ($P<0.05$) of the added solution than BF. Injected steaks had significantly ($P<0.05$) higher fluid loss after 48 h but retained more fluid after thawing compared to non-injected samples.

Table 3.1 Thaw loss, pH and brine retention after 48 h

	Purge@48h (%)	Thaw loss (%)	pH
Grade			
D2	1.87	2.64	5.56
Y1	1.63	3.34	5.56
SEM	0.21	0.22	0.01
P	0.43	0.07	0.68
Muscle			
BF	1.34b	3.15ab	5.54b
L	2.05a	3.44a	5.63a
SM	1.87a	2.39b	5.51b
SEM	0.17	0.26	0.01
P	0.00	0.03	0.00
Treatment			
C	1.04c	3.37a	5.47b
ME	2.64a	2.70b	5.65a
BT	1.04c	3.14a	5.49b
BT+ME	2.29b	2.77b	5.62a
SEM	0.17	0.19	0.02
P	0.00	0.00	0.00

a-c, Means with different letters in the same column (within each main effect) are significantly different ($P<0.05$).
Biceps femoris (BF). *Longissimus* (L). *Semimembranosus* (SM).
Tenderization treatments: control (C), steaks injected with salt/phosphate solution (ME), blade tenderized (BT), blade tenderized followed by injection with salt/phosphate solution (BT+ME).
SEM - standard error of the mean.

The meat from youthful animals had lower cooking losses than those from mature animals, whereas SM muscle had the highest cooking loss among three muscles followed by BF and L having the lowest cooking loss (Table 3.3). The cooking loss was greatly influenced by the method of cooking (i.e. dry- or moist-heat). Grilling of steaks produced lower cooking losses but only for not treated and moisture enhanced steaks. There was no difference between cooking methods in cooking loss for blade tenderized steaks.

Moisture enhancement had a beneficial effect on overall yield of cooked samples. Cooking yield expressed as a percentage of green weight for injected samples was significantly ($P < 0.05$) higher compared to non-injected muscles. Increasing pH has been reported to improve cooking yields (Bouton, Harris, & Shorthose, 1982); the pH of injected muscle sections was significantly ($P<0.001$) higher than non-injected samples. Boles and Shand (2001) and Boles and Swan (1996) have also reported a significant increase in cooking yield in roast beef with brine injection. Cooking by moist-heat resulted in significantly ($P<0.001$) higher cooking losses compared to cooking by dry-heat. Increased moisture retention (higher EM –Table 3.3) during grill cooking was the most probable reason for a higher cooking yield compared to moist-heat cooking.

Table 3.3 Water binding properties and hardness.

	Cook loss (%)	Yield (%)	EM (%)	Hardness (N)
Grade				
D2	30.98a	69.86	12.30b	56.08
Y1	29.57b	70.90	13.53a	53.05
SEM	0.42	0.70	0.23	2.53
p	0.00	0.31	0.00	0.42
Muscle				
BF	30.90b	69.53b	12.30b	51.45b
L	27.94c	72.71a	13.13a	40.26c
SM	31.99a	68.90b	13.32a	71.98a
SEM	0.36	0.59	0.28	2.34
p	0.00	0.00	0.04	0.00
Treatment				
C	30.20ab	67.01b	11.97c	62.30a
ME	29.71a	74.57a	14.53a	53.27b
BT	30.75b	66.29b	11.55c	54.33b
BT+ME	30.45ab	73.65a	13.61b	48.37b
SEM	0.38	0.61	0.29	2.40
p	0.05	0.00	0.00	0.00
Cooking				
Grill	29.20b	71.56a	15.15a	51.05b
Moist	31.35a	69.20b	10.69b	58.08a
SEM	0.33	0.51	0.21	1.99
p	0.00	0.00	0.00	0.00

a-c, Means with different letters in the same column (within each main effect) are significantly different ($P<0.05$).
Biceps femoris (BF); Longissimus (L); Semimembranosus (SM)
Tenderization treatments: control (C), steaks injected with salt/phosphate solution (ME), blade tenderized (BT), blade tenderized followed by injection with salt/phosphate solution (BT+ME).
SEM, standard error of the mean.

Expressible moisture (EM) is a measure of free water in the cooked product and may be an indicator of purge to be expected in the packaged product (Shand, 2000). Grilled steaks from muscles obtained from D2 mature cattle had significantly lower EM than did steaks from youthful animals, but no difference between grades in moisture retention was seen in moist cooked steaks. Injected steaks had significantly higher EM than beef steaks made from non-injected meat indicating that there is more free moisture in the product as a result of brine addition. Moist cooking resulted in a lower percentage of water loss from meat samples compared to grilling, thereby indicating lower amounts of free moisture due to larger moisture loss during cooking.

3.3.2 Warner Bratzler Shear Force (WBSF)

As expected, muscles from youthful cattle had significantly lower WBSF values than muscles from mature animals (Table 3.4). Regardless of cooking method, the L muscles had the lowest shear force, whereas WBSF values for BF and SM depended on cooking regime. BF was significantly toughest when grilled but there was no significant difference in WBSF between SM and BF muscles when steaks were cooked using a slow moist regime. This observation suggested that the impact of cooking method on tenderness was dependent on muscle type. In fact only BF steaks cooked by moist method were more tender (lower WBSF values) than grilled samples, while L and SM steaks did not show added benefit of moist heat cooking. Different responses to cooking regimes may be due to the differences in the connective tissue content between *semimembranosus, longissimus* and *biceps femoris*.

Slow moist cooking drastically reduced SF-C (connective tissue component of WBSF) in BF steaks and slightly in SM steaks. However, no reduction in SF-M (myofibrillar component of WBSF) shear force was observed with moist cooking. Cooking regime had also no significant effect on hardness giving further evidence of the role of connective tissue in tenderness.

3.4 Conclusion

All tenderization treatments significantly reduced WB shear force values compared to non-injected steaks. Moisture enhancement and blade tenderization resulted in a reduction of WBSF by 10 N (about 1 kg) compared to the control and were equal in their tenderizing effect. However, BT followed by injection with a salt and phosphate solution resulted in the lowest WBSF values and was the most effective strategy for improving tenderness of all muscles. Additional reduction in shear force observed with BT in moisture enhanced muscles, suggests that the combination of mechanical tenderization with the moisture enhancement may provide a useful means of improving tenderness of beef to more acceptable levels. Regardless of cooking method, the L muscles had the lowest shear force, whereas WBSF values for BF and SM depended on cooking regime. BF was significantly toughest when grilled but there was no significant difference in WBSF between SM and BF muscles when steaks were cooked using a slow moist regime. This observation suggested that the impact of cooking method on tenderness was dependent on muscle type. In fact only BF steaks cooked by moist method were more tender (lower WBSF) than grilled samples, while L and SM steaks did not show added benefit of moist heat cooking. Different responses to cooking regimes may be due to the differences in the connective tissue content between *semimembranosus, longissimus* and *biceps femoris*.

Table 3.4 Warner-Bratzler shear force value means

	WBSF (N)	SF-C (N)	SF-M (N)
Grade			
D2	78.9a	69.1a	69.5a
Y1	52.3b	39.6b	49.5b
SEM	2.32	3.09	1.88
P	0.00	0.00	0.00
Muscle			
BF	77.2a	72.3a	64.8a
L	48.5b	31.6c	47.7b
SM	71.0a	59.0b	66.0a
SEM	2.72	2.92	2.30
P	0.00	0.00	0.00
Treatment			
C	75.7a	59.0a	70.4a
ME	65.0b	58.1a	55.4c
BT	67.3b	53.8a	62.5b
BT+ME	54.3c	46.3b	49.6d
SEM	2.45	2.78	1.98
P	0.00	0.00	0.00
Cooking			
Grill	69.3a	60.0a	60.3
Moist	61.8b	48.7b	58.6
SEM	1.74	2.27	1.41
P	0.00	0.00	0.27

a-c, Means with different letters in the same column (within each main effect) are significantly different ($P<0.05$).
WBSF, peak shear force value. SF-C, connective tissue component of WBSF; SF-M, myofibrillar component of WBSF (N = newton, SI unit of force), a newton can be converted into kg force by dividing the N value by 9.80665.
Biceps femoris (BF). *Longissimus* (L). *Semimembranosus* (SM)
Tenderization treatments: control (C), steaks injected with salt/phosphate solution (ME), blade tenderized (BT), blade tenderized followed by injection with salt/phosphate solution (BT+ME).
SEM, standard error of the mean.

Personnel Involved

Z. Pietrasik, P.J. Shand, H. Silcox and N. Wiebe

Funding:

The Beef Cattle Research Council, with funding support from the Canadian Cattlemen's Association, provided the meat for this project. ADF and BDF funding supplied analysis costs and a portion of the salary of Dr Z. Pietrasik.

References

References cited in the text are available on request.

Communication of results:

A manuscript based on this work is being prepared for submission to peer reviewed journals. The results were also communicated to the industry through oral presentations and a factsheet developed by the Beef Information Centre

Acknowledgements

Thanks are expressed to Natural Valley Farms Inc for their assistance in collection of meat samples for part of this study. Some meat for this study was also obtained through funding provided by the Canadian Cattlemen's Association through the Beef Information Centre. Part of Dr. Pietrasik and H. Silcox's salary was also provided by the Value Added Beef Program (Saskatchewan Beef Development Fund).

Personnel funded by this project

H. Silcox (30% of time) and A. Perera (partial grad student stipend - 50%)

Personnel contributed to the project

Zeb Pietrasik, P.J. Shand, Neil Wiebe and D. Pobereznek.

Activity 4: Evaluate the efficacy of chemical treatments on properties of connective tissue/meat cuts from mature animals

4.1 Introduction

The contribution of connective tissue to beef toughness was extensively investigated (Sifre et al., 2005; Purslow, 2005; Lepetit, 2007). Perimysial and endomysial collagen content and collagen fibre diameter were reported to correlate with beef toughness (Light et al., 1985). Meat tenderization treatments are generally applied onto the meat matrix in which connective tissue and muscle fibres are interconnected together. As a result, the effect of tenderizing agents on the contributors to toughness and particularly on connective tissue itself is not elucidated.

Alkalis are not widely used as meat tenderizers except for some attempts to use sodium bicarbonate (Sheard and Tali, 2004) and ammonium hydroxide (Hamling and Calkins, 2008). Sodium hydroxide is widely used in the production of gelatine from animal sources but not as a meat tenderizer. Also, there is evidence that toughness (shear stress) of epimysium from youthful cattle (heifers) can be substantially decreased through aqueous heating but epimysium from mature cattle (cows) remains tougher before and after aqueous heating [Perera, 2009]. In this study, cow epimysium was used as a model to assess the potential to utilize sodium hydroxide and ammonium hydroxide as connective tissue tenderizers and to understand the impact of alkali on connective tissue properties.

To reduce the connective tissue driven toughness, beef was marinated with organic acids such as acetic, lactic and citric (Arganosa and Marriott, 1989; Aktas and Kaya, 2001; Berge et al., 2001). The application of acids to tenderize meat might be linked to their ability to solubilize collagen (Bowes et al., 1955; Kruggel, et al., 1970; Whiting and Strange, 1990; Meyer, 2003). Acetic acid (CH3COOH) *per se* was often used for the isolation of collagen from tissues (Maekawa et al, 1970; Rhodes and Miller, 1978; Nagai and Suzuki, 2000).

4.2 Materials and methods

4.2.1 Epimysium sample preparation

Six *longissimus* muscles from cows of utility grade were purchased and stored at 4 °C. On the 10th day post-mortem, dorsal epimysium was excised and stored at -20 °C. Prior to use, visible fat and muscle fibres were removed and the epimysium was cut into two strips (width 2 cm) along the length. From the strips, pieces of 2x2.5 cm were prepared. Treatments were applied in a split-plot design, temperature as the main plot and concentration of alkali or acid and pre-equilibration time as sub-plots. This experiment was repeated with epimysium from three different animals.

Epimysium pieces were pre-equilibrated (0, 90, 180 min) in 10 mL of aqueous sodium hydroxide (0.01, 0.025, 0.05 M; pH 12.0-12.7) and aqueous ammonium hydroxide (0.1, 0.25, 0.5 M; pH 11.1-11.4) prior to heating at 55 °C and 70 °C for 15 min. As a control, epimysium pieces from the same animals were heated in

de-ionized water (10 mL) for 15 min at 70 °C. Acid treatments were applied in a similar manner to alkalis. Each epimysium piece within a temperature treatment was immersed in 10 mL of HCl or CH_3COOH (0.1, 0.25 and 0.5M) concentrations. The pH values of HCl and CH_3COOH solutions were in the ranges of 1.10-1.54 and 2.99-3.21, respectively.

4.2.2 Protein determination

Protein released from epimysium was measured using the bicinchoninic acid (BCA) assay procedure, as already explained in activity 2.

4.2.3 Epimysial shear stress

Epimysial shear stress before and after heating with alkali was determined as explained in activity 2. The maximum shear stress generated was expressed as the force applied on a unit cross sectional area of sample and also corrected for raw thickness (N/mm^2).

4.2.4. Thermal denaturation temperatures

Thermal denaturation temperatures of epimysial proteins were determined. Briefly, raw epimysium from 6 cows was first cut into small pieces and then ground after freezing in liquid N_2. About 0.5 g of ground epimysium was suspended in 10 mL of de-ionized water (control), 0.25 M NH_4OH and 0.025 M NaOH. Samples were equilibrated overnight at 4 °C. Excess liquid was drained out and epimysium samples were briefly dried on filter paper. Samples (7-8 mg) were weighed into coated aluminium pans and hermetically sealed with lids. Pans were heated from 20 to 140 °C using a differential scanning calorimeter (DSC Q 2000, TA Instruments, USA) at a rate of 5 °C/min. Sealed, empty pans were used as the reference. Data analysis was carried out using curve integration software (TA Universal Analysis 2000).

4.2.5 Infrared spectroscopy

IR spectra of amide bands of epimysial proteins were determined. Epimysium pieces (2x2.5 cm) were suspended in 10 mL of de-ionized water (control) and 0.5 M NH_4OH (180 min pre-equilibration). In a pre-heated water bath, the internal temperatures of samples were brought to 70 °C and heating continued for 15 min. Samples were stored at -20 °C. Selected samples were sectioned, placed in plastic moulds and filled with embedding compound OCT cryomatrix® (Shandon Company, USA). The preparation was frozen in liquid nitrogen and stored at –20 °C. Serial sections (6 μm) were cut from cryo-preserved samples using a cryo-microtome. Sections were placed on reflective-coated Low-e® microscope slides (Kevley Technologies, USA) for spectroscopic analysis. FTIR imaging was conducted using 01B1-1 Mid IR beam line (800-2000 cm^{-1}) at the Canadian Light Source. Spectroscopic mapping was performed in reflection mode with diffraction-limited spatial resolution. For each pixel, 64 scans, with a 4 cm^{-1} spectral resolution, were co-added. Data analysis was conducted using OPUS software (Bruker Optics, Germany).

4.2.6 Statistical analysis

PROC-GLM of SAS 9.1 programme was used for the statistical analysis of data.

4.3 Results and Discussion

Moisture, protein and fat levels of the epimysium samples used are presented in Table 4.1. Raw epimysium samples had shear stress values of 38±7 N/mm^2.

Table 4.1 Properties of cow epimysium, (N = 18).

Property	Mean ± SD
Moisture (g/100g wb)	55.2±1.7
Protein (g/ 100g wb)	45.6±3.7
Fat (g/100g wb)	3.9±1.6
Shear stress (N/mm^2)	38.4±6.8

4.3.1 Protein released

After alkali treatments, the maximum amount of protein released was <2% of the weight of epimysium or <5% of epimysial proteins. Following 180 min pre-equilibration in sodium hydroxide (0.01-0.025 M) and subsequent heating to 55°C, single (α) and double strands (β) of collagen were liberated (Figure 4.1a). As the concentration of sodium hydroxide (0.05 M) and temperature (70 °C) were increased, collagen peptides were hydrolysed. Ammonium hydroxide also had liberated single and double strands of collagen at the two temperatures investigated (Figure 4.1b). Some hydrolysis of collagen peptides was noted after 0.5 M ammonium hydroxide treatment (180 min pre-equilibration) followed by heating to 70 °C.

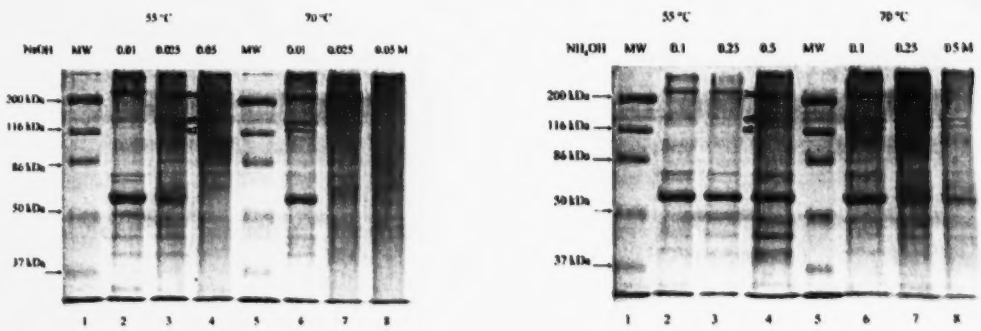


Figure 4.1 Peptides released from epimysium following 180 min pre-equilibration in alkali and heating at 55 and 70 °C for 15 min. (a) Lanes 2, 3, 4 (55°C) and lanes 6, 7, 8 (70°C) show sodium hydroxide concentrations 0.01, 0.025 and 0.05 M, respectively. (b) Lanes 2, 3, 4 (55°C) and also lanes 6, 7, 8 (70°C) show ammonium hydroxide concentrations 0.1, 0.25 and 0.5 M, respectively. Lane 1 and 5 are molecular weight standards (kDa). α1 and α2 represent single strands of collagen and β represents double strands of collagen.

The amounts of protein released from epimysium subsequent to acid treatments are presented in Figure 4.2 (a-d). Protein release was influenced by the increasing concentration of acid, pre-equilibration time in acid before heating and mostly by the temperature of heating. As expected, pre-equlibration treatment of 90 and 180 min in 0.5M HCl had significantly solubilised the epimysial proteins (Figure 4.2b). Acetic acid at the highest concentration (0.5M) had solubilised less than 3g proteins/100g epimysium (Figure 4.2d)

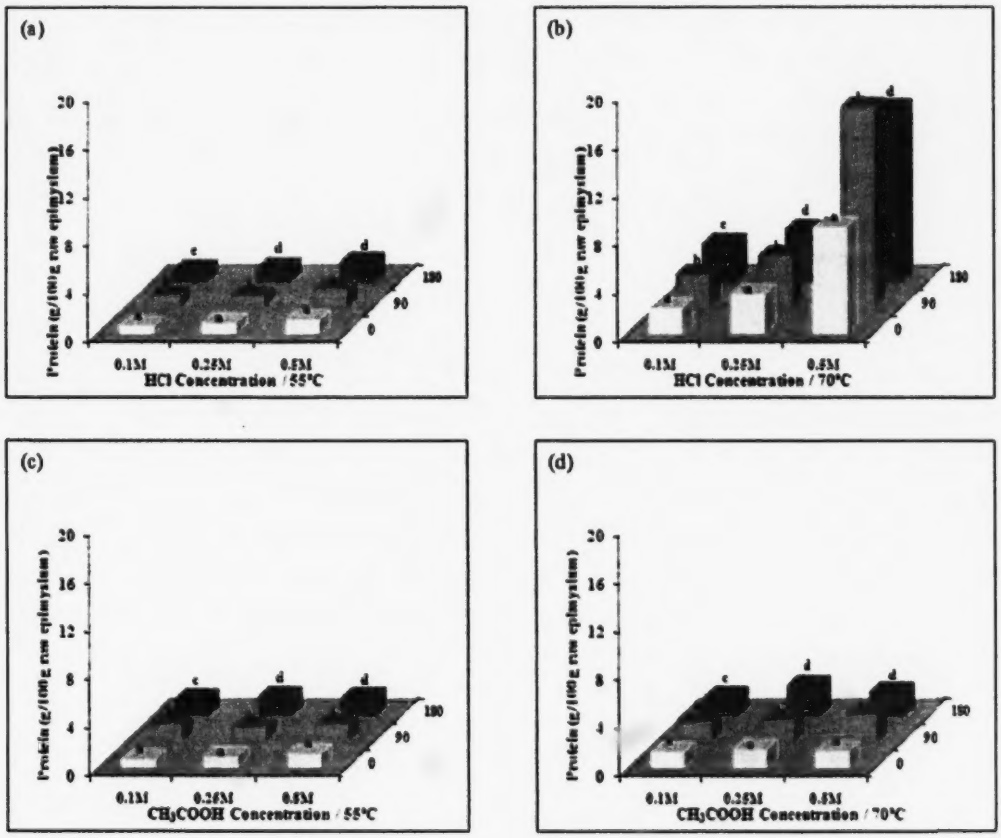


Figure 4.2 Protein release data of epimysium after acid treatments. (a) Heating in HCl at 55°C. (b) Heating in HCl at 70°C. (c) Heating in CH_3COOH at 55°C. (d) Heating in CH_3COOH at 7°C.

4.3.2 Shear stress

Shear stress values of epimysium (raw 38 N/mm^2) was decreased to ~25-29 N/ mm^2 as samples were pre-equilibrated (0-180 min) in sodium hydroxide (0.01-0.025 M) and subsequently heated to 55°C (Figure 4.2a and 4.2b). The decrease in shear stress is attributed to the effect of temperature (55°C) because samples heated in water had reached similar values (~23 N/mm^2). As the concentration of sodium hydroxide was increased to 0.05 M, shear stress was further decreased by10 N/mm^2. Ammonium hydroxide concentration

(0.1-0.5 M) and pre-equilibration (0-180 min) in it did not decrease shear stress (~39 N/mm^2) at 55°C (Figure 4.2c and 4.2d). The effect of temperature (55°C) in decreasing shear stress was eliminated by ammonium hydroxide treatment because samples heated in water had reached 24 N/mm^2. Heating at 70°C had a significant effect on epimysial shear stress reduction. Shear stress of sodium hydroxide and ammonium hydroxide treated epimysium was decreased to 3-6 N mm^2 after heating at 70°C and which was similar to that heated in water (~5 N/mm^2). The epimysium stabilizing effect of ammonium hydroxide was eliminated at70°C.

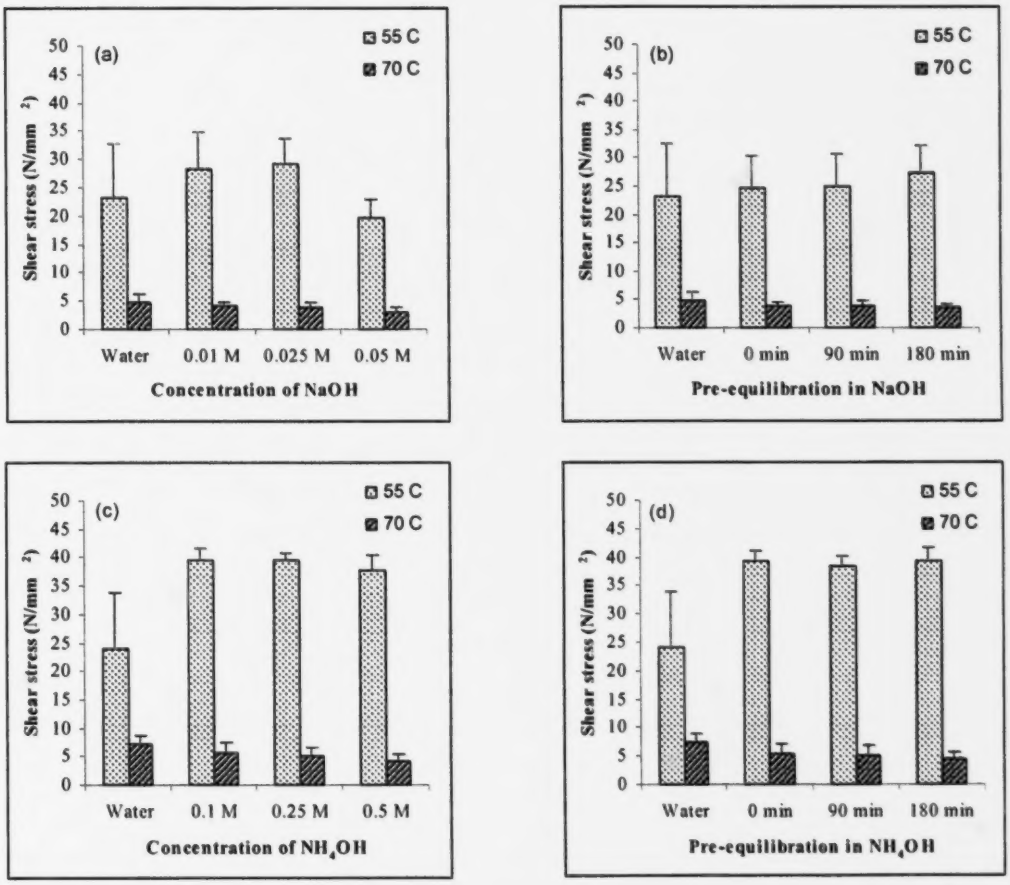
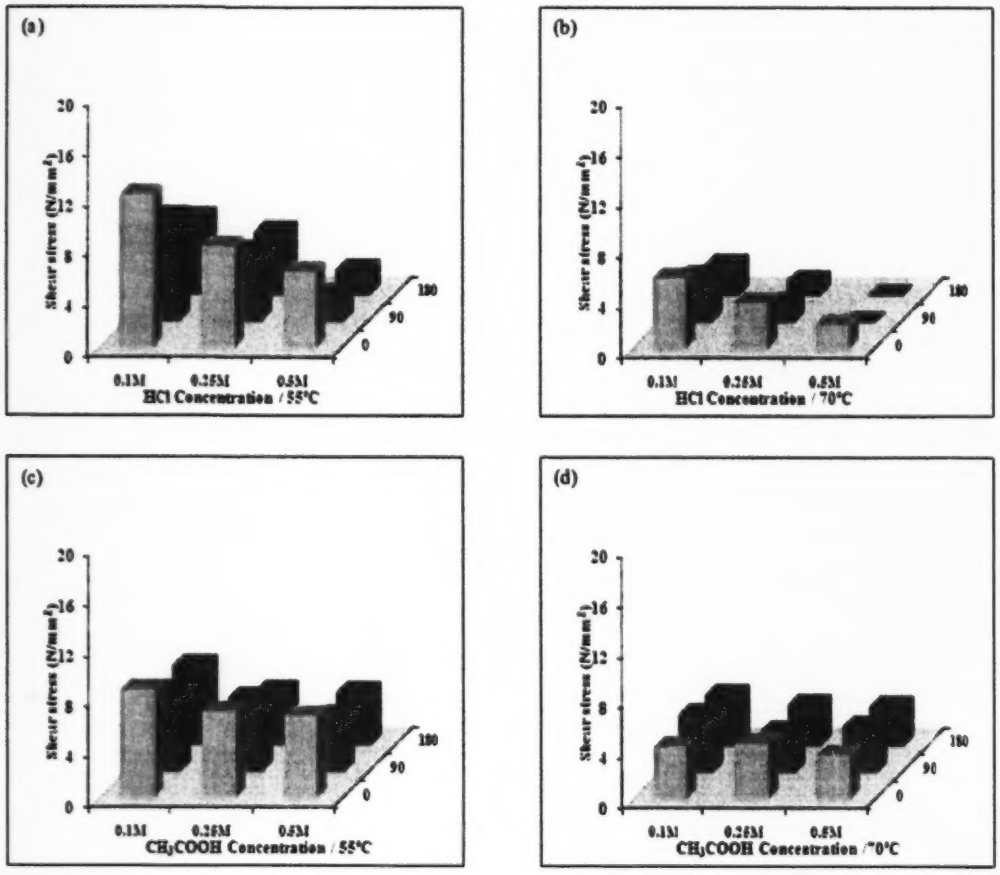


Figure 4.2 Changes in epimysial shear stress (means ± SE) following alkali treatments (a) Effect of the concentration of ammonium hydroxide and temperature. (b) Effect of the pre-equilibration time in ammonium hydroxide and temperature. (a) Effect of the concentration of sodium hydroxide and temperature. (b) Effect of the pre-equilibration time in sodium hydroxide and temperature.

Shear stress values of acid treated epimysium are presented in Figure 4.3 (a-d). The increasing concentration of acid, pre-equlibration time in acid and temperature of heating had significantly contributed to the decreased shear stress values. The effect of HCl is more prominent at 70°C where at 0.25M concentration shear stress decreased to < 3 N/mm^{2}and at 0.5M concentration of acid epimysium was mostly solubilzed and shear stress could not measure (Figure 4.3b). At 0.25 and 0.5M concentrations and at 70°C, acetic acid was effective in decreasing epimysial shear stress < 5 N/mm^2.



4.3.4 Thermal denaturation temperature

Epimysium samples saturated in water prior to heating had a thermal denaturation temperature of ~63 °C (Table 4.2). At the concentration used (0.025 M), sodium hydroxide did not change the melting temperature of epimysial proteins (~64 °C). Following ammnonium hydroxide treatment (0.25 M), denaturation temperature was significantly increased to ~66 °C with a narrower and a deeper denaturation curve compared

to other treatments. This observation supports the hypothesis that ammonium hydroxide has a structure stabilization effect on collagen because ~91% of the epimysial proteins consisted of collagen (Perera, 2009).

Table 4.2 Melting temperatures (mean ± SE) of epimysial proteins after aqueous and alkali heating

	Melting temperature peak (°C)	Onset temperature (°C)
Water	63.0 ± 0.5	53.3 ± 0.6
0.025 M NaOH	64.0 ± 0.3	55.9 ± 0.6
0.25 M NH_4OH	66.2 ± 0.1	55.9 ± 0.5
P	0.05	0.05
LSD	2.00	2.18

Heating carried out at 5 °C/min from 20-140 °C. Each treatment had 6 replicates and three sub samples.

4.3.5 Infra red spectrum

After aqueous heating of epimysium (from animals <30 months and 3-5 years) at 70-95 °C, 14-16 g amorphous proteins in 100 g epimysium (or ~30% on a protein basis) was extracted with pronase (Perera, 2009) indicating helix→coil transition. However, aqueous heating at 70 °C (15 min) did not introduce changes to the typical amide bands I (1675-1658 cm^{-1}) and II (1550 cm^{-1}) of epimysial proteins (Figure 4.3a). Therefore, C=O and N-H bond stretching (Pelpis et al., 1996) might not have happened in collagen peptides during heating at 70 °C for 15 min, even though some of the epimysial collagen converted to an amorphous state under similar conditions of heating (Perera, 2009) or else, significant recoiling of collagen (coil→helix transition) (Hörmann and Schlebusch, 1971) might have happened during cooling. A new peak emerged (1640-1620 cm^{-1}) on the shoulder of the amide I band of ammonium hydroxide treated and subsequently heated epimysium (Figure 4.3b). This band represents random coils (Martinez and Millhauser, 1995) and also β sheets (Byler and Susi, 1986). Because glycine contributes to ⅓ of amino acids of major epimysial protein collagen (Estoe (1967) it is thought this new peak represents random coils (Smith et al., 1994). Perhaps, ammonium hydroxide has prevented or decreased the coil→helix transition of thermally denatured collagen.

4.4 Conclusion

Sodium hydroxide (0.05 M) tenderizes connective tissue more than water at 55°C but at 70°C, water is an equally good tenderizer as alkali. Ammonium hydroxide (0.1- 0.5 M) had no useful tenderizing effect on connective tissues. Instead, ammonium hydroxide had a collagen stabilization effect and raised the melting temperature of collagen. Acids tenderize epimysium through solubilising proteins and the effect of acid is more prominent at 70°C than at 55°C. Pre-equlibration of epimysium in acid prior to heating helps to further decrease shear stress than immediately heating in acid.

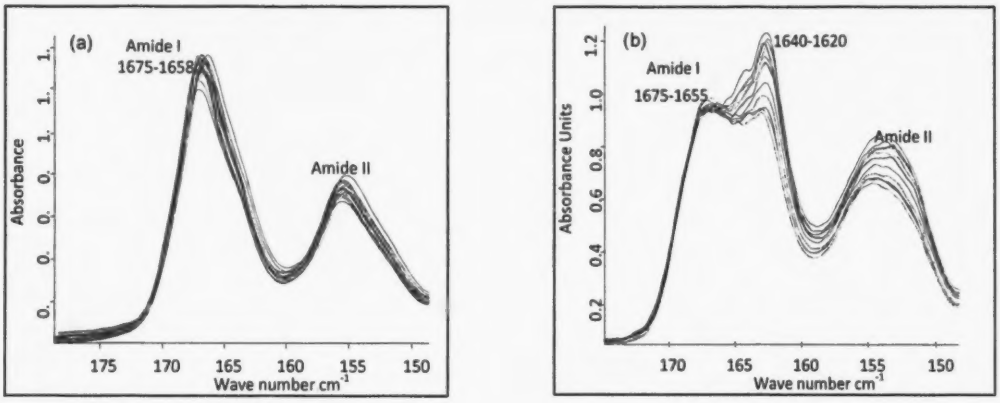


Figure 4.3 IR spectra of epimysium. (a) Heated to 70 °C with de-ionized water with no pre-equilibration. Amide bands I (1675-1658 cm^{-1}) and II (1550 cm^{-1}) typical to proteins are observed despite the exposure to high temperature. (b) Pre-equilibrated for 180 min in 0.25 M NH_4OH and heated to 70 °C for 15 min. On the shoulder of the characteristic amide I band (1675-1655 cm^{-1}) a new peak emerged (1640-1620 cm^{-1}).

References

Will be provided on request

Communication of results

Perera*, A., Pietrasik, Z. and **Shand, P.J.** 2008. Effect of strong and weak acids on the structural integrity of bovine epimysium. Institute of Food Technologists Annual Meeting, New Orleans, LA. Abstract, page ?. [poster A.P.] *graduate student

Perera*, A., Pietrasik, Z. and **Shand, P.J.** 2009. Effect of strong and weak acids on the structural integrity of bovine epimysium. Canadian Meat Science Association / Canadian Meat Council Joint Technical Symposium, May 7, Montreal, Quebec. Abstract-proceedings. [poster PJS] *graduate student

Perera*, A., Olkowski, A.A. and **Shand P.J.** 2009. Efficacy of sodium and ammonium hydroxide as connective tissue tenderizers. Proceedings of the 55th International Congress of Meat Science and Technology, Copenhagen, Denmark, August 16-21. PE7.16 pp. 1141-1146. [short paper and poster]*Ph.D. student

A manuscript is being prepared and to be published in a peer reviewed journal.

Acknowledgement

The Canadian Cattlemen's Association and the Agriculture Development Fund of the Saskatchewan Ministry of Agriculture funded this project.

Personnel contributed to the project

Anula Perera (PhD student), Dr Andrew Olkowski, Dr. P.J. Shand and D.Pobereznek.

Activity 5: Investigation of process methods for chopped and formed beef rolls

5.1 Introduction

The objectives of this study were to compare the sensory, textural properties of restructured beef (a) made with meat obtaining from two age groups under thirty months (UTM) and over thirty months (OTM), made with three different meat flakes sizes in making restructured meat logs, and cooked in a smokehouse with and without relative humidity.

5.2 Materials and Methods

5.2.1Meat preparation

Inside beef rounds (*semimembranosus* and *gracilus* muscles) from Canadian Grade A carcasses were obtained from XL Meats, a federally inspected facility located in Alberta. Two age groups of inside beef rounds were tested in this study, from under thirty months old cattle (UTM beef); and over thirty months old cattle (OTM beef). They were individually packaged in vacuum bags, received and stored at refrigerated temperature, and tempered at -2°C cooler for 16-18 hours before being processed. Visible fat and connective tissues from the surface of the fresh meats were trimmed off (Figure 5.1a and 1b). Each group of inside rounds was cut into 4-6 cm cubes and further reduced in size using the flaking method (Figure 5.1c). Meats were flaked through a Model 3600 Urschel Comitrol (Urschel Laboratories, Inc., Valparaiso, IN) that is equipped with 3 different Comitrol heads: 3K030180D (4.6mm oper[illegible]); 3K030300U (7.6mm opening); or 3J030510D (13mm opening) (Figure 5.2a). Flaked meat (Figure 5.2b[illegible]rature was between 0°C to -1°C.

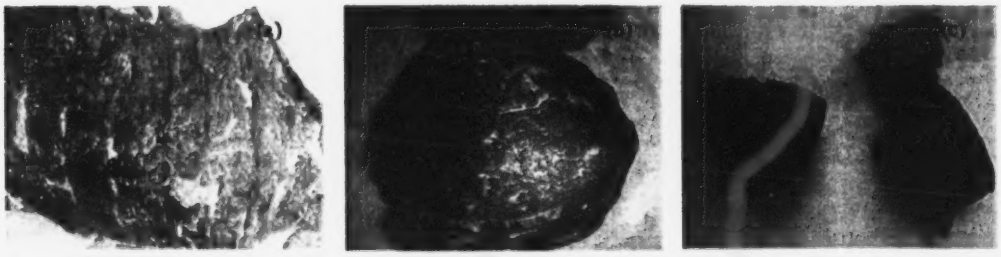


Figure 5.1 Inside round meat used in this study. (a) OTM beef. (b) UTM beef. (c) Cubed beef (left-OTM and right-UTM)

5.2.2 Product preparation

UTM and OTM meat having three different flakes sizes were labeled as 'S' for small flakes, 'M' for medium flakes and 'L' for large flakes. Flaked meat was mixed with 3% water, 1% salt, 0.35% sodium tripolyphosphate in a Hobart mixer-Model H60 (the Hobart Mfg. Co. Ltd., Don Mills, ON) at speed 1 for 2.5 minutes. The raw seasoned small flake mixture was brought to a tightly bound state and formed into a ball at the mixer blade. The raw seasoned large flake mixture was brought to a loosely bound state during the standardized mixing time. The mixtures were then stuffed into No: 8 fibrous casings (Viscofan Canada,

Quebec) using a Handtmann VF20 vacuum stuffer (Albert Handtmann, West Germany). From each comitrol flake size, two logs of meat mixture were prepared. Six samples of 100g seasoned meat mixture were randomly collected (2 groups of 3 meat flake sizes) for proximate analysis and pH determination.

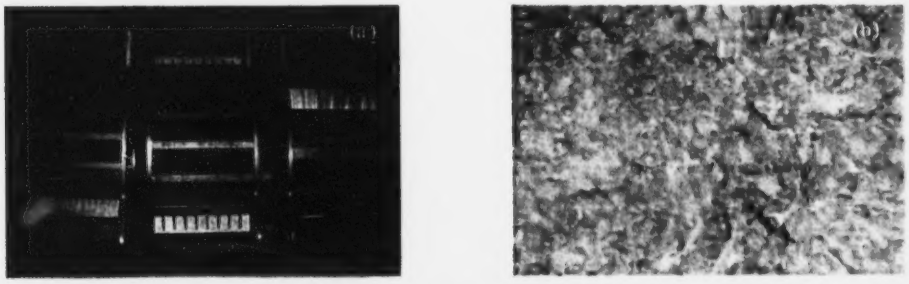


Figure 5.2 (a) Comitrol heads (left to right) 3K030180D (4.6 mm opening); 3K030300U (7.6 mm opening); 3J030510D (13 mm opening). (b) Comitrol meat flakes.

5.2.3 Cooking method and cooked yield

Meat logs were cooked in an Alkar-2000 smoke oven (ALKAR-RapidPak, Inc., Wisconsin). Two methods of cooking were employed. One set of meat logs was cooked using a fast cooking cycle (cooking method No: 1) and the second set was cooked using a slow cooking cycle (cooking method No: 2).

In the cooking method No: 1, meat logs were cooked at 85% relative humidity and with three stages of incremental temperature increases (40, 60 and 85°C) to reach the internal temperature to 76°C. A similar method was used in the cooking method No: 2, except that relatively humidity was maintained at 0%. All meat logs were weighed before and after cooking. Cooked yield was determined as [(the weight of cooked samples / the weight of the raw samples) x 100].

5.2.4 Salt determination

Salt content of cooked meat logs were determined using HACH salt analysis (HACH Company, Ontario).

5.2.5 Sensory evaluation results

Cooked meat logs were packaged in vacuum bags and stored at 4°C. Meat logs were sliced before use and served cold. A three digit code was assigned to each sample treatment. Samples were presented to panelists in a random order. Ten panelists were instructed to score on appearance, colour, juiciness, chewiness, flavour and overall acceptability using a 6-point hedonic scales (1= very unacceptable; 6= very acceptable).

5.3 Results and discussion

5.3.1 Cooked yield

Images of cooked meat logs representing all treatments are presented in figure 5.3. Cook yield was not affected by the source of meat, i.e. animal age expressed as OTM and UTM. Meat logs made with larger meat flakes had shown the lowest cook yield (Table 5.1). Meat logs made using medium and small meat flakes had similar cook yields which were higher than that from large meat flakes. It was speculated that more protein was being extracted in the samples made with smaller meat flakes, thus increased the meat binding ability and cooked yield.

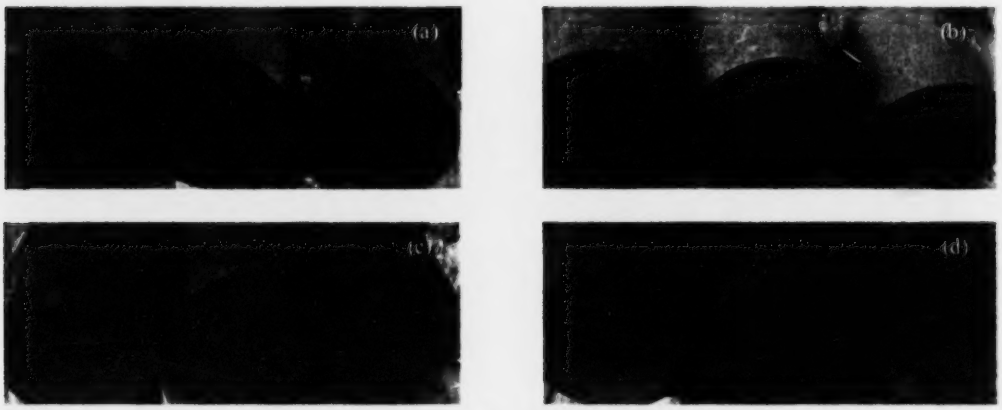


Figure 5.3 Cooked meat logs (a) Cooking method No: 1, OTM beef - left to right: large (ML1), medium (MM1) and small (MS1) flakes of meat. (b) Cooking method No: 1, UTM beef - left to right: large (YL1), medium (YM1) and small (YS1) flakes of meat. (c) Cooking method No 2, OTM beef - left to right: large (ML2), medium (MM2) and small (MS2) flakes of meat. (d) Cooking method No: 2, UTM beef - left to right: large (YL2), medium (YM2) and small (YS2) flakes of meat.

5.3.2 Shear force

Shear force data of meat logs were similar between two groups of animals, UTM and OTM. Also, differences in sizes of meat flakes, i.e. large, medium and small, did not influence shear force values of the processed product.

5.3.3 Sensory data

Meat samples originating from both UTM and OTM, having different meat flake sizes, and having beem cooked as per method No: 1 and No: 2 were similar in juiciness and flavor (Table 5.2). However, meat samples made with OTM beef were relatively juicier than samples made with UTM beef when samples were prepared according to cooking method No: 1. Samples made with medium and large meat flakes had better flavour than samples made with small meat flakes. Sensory scores obtained from the two different cooking methods were compared. Samples cooked in method No: 1 (slow cooking with humidity) scored high in

appearance and colour. Meat samples made with OTM beef and cooked with humidity scored high in colour, juiciness and overall acceptability than samples cooked without humidity.

Table 5.1 Salt content, cooked yields and shear force data of meat logs

Sample	Treatment	Salt %	Cooked yield %	Shear force (N)
YS1	UTM beef, small meat flakes, fast cooking method 1	1.34	89.45	23.9
YM1	UTM beef, medium meat flakes, fast cooking method 1	1.37	89.71	26.6
YL1	UTM beef, large meat flakes, fast cooking method 1	1.45	85.71	25.5
MS1	OTM beef, small meat flakes, fast cooking method 1	1.21	90.79	32.1
MM1	OTM beef, medium meat flakes, fast cooking method 1	1.24	88.89	28.0
ML1	OTM beef, large meat flakes, fast cooking method 1	1.45	86.99	22.7
YS2	UTM beef, small meat flakes, slow cooking method 2	1.30	89.46	23.7
YM2	UTM beef, medium meat flakes, slow cooking method 2	1.36	89.31	22.4
YL2	UTM beef, large meat flakes, slow cooking method 2	1.31	84.95	22.7
MS2	OTM beef, small meat flakes, slow cooking method 2	1.21	88.00	27.2
MM2	OTM beef, medium meat flakes, slow cooking method 2	1.38	87.73	24.7
ML2	OTM beef, large meat flakes, slow cooking method 2	1.24	79.86	24.7

Salt% = Average (from duplicates) salt content of cooked meat
Cooked yield % = [(cooked weight / raw weight) *100]
OTM - Beef from animals that were over thirty months old
UTM - Beef from animals that were under thirty months old

	[1]Sensory scores					
Sample ID	Appearance	Colour	Juiciness	Chewiness	Flavour	Overall acceptability
YS1	4.0	4.2	2.3	3.3	2.3	2.5
YM1	4.2	4.3	2.8	3.5	3.3	3.0
YL1	4.4	4.5	3.0	3.2	3.2	3.0
MS1	4.2	4.5	3.4	3.6	2.7	3.5
MM1	4.3	4.6	3.7	3.5	3.3	3.7
ML1	4.1	4.7	4.1	3.6	3.4	3.6
YS2	3.7	4.0	2.5	3.1	2.8	2.5
YM2	4.0	4.3	2.9	3.4	3.4	3.1
YL2	3.8	4.0	3.1	3.7	3.3	3.4
MS2	3.7	3.7	3.1	2.1	3.0	2.5
MM2	4.0	4.1	3.1	3.9	3.2	3.2
ML2	4.3	4.5	3.8	3.8	3.4	3.3

1 = Average sensory scores obtained from 10 panelists

5.4 Conclusion

The product quality of meat logs is not sensitive to the age of the animal, < 30 months and > 30 months, which indicated that if muscles are properly trimmed of exterior connective tissue, commercial beef would be a good source of meat for restructured products. Cooked yield is improved by using medium and small sizes of flakes. As flavour of the final product is high with medium and large size flakes but low with small flakes it is concluded that medium flakes produce the meat logs with the most favourable quality. Cooking in humid conditions improves the juiciness of the final product. This project was carried out with a limited number of panelists. Evaluation of these products by a larger consumer panel would be desirable in a future study.

Acknowledgement

Services of Saskatchewan Food Industry Development Centre are appreciated in carrying out the study.

Personnel contributing to the project

Sara Lui, B. Chesky, Saskatchewan Food Industry Development Centre.

Dr. P.J. Shand and H. Silcox

Members of the informal sensory panel

Appendix 1: Other Activities

During 2008, Dr. Lepetit from INRA, France, a recognized expert on collagen, spent a two month research leave with my research group. During that time, he contributed to our collagen research and developed a new technique for evaluation of collage fibers. A paper presented at the International Congress of Meat Science and Technology is reproduced below.

6.1 Effect of Heating on the Transverse Adhesion of Collagen Fibers by J. Lepetit[1] and P. J. Shand

[1]J. Lepetit was with the National Institute for Agricultural Research, Unit of Quality of Animal Products, 63122 Saint Genès Champanelle, France. (now deceased)

6.1.1 Abstract—The mechanical properties of a composite fibrous material depend on the properties of the fibers, of the matrix and of the interactions between them. The purpose of this work was to investigate the evolution of the adhesion between collagen fibers and the extracellular matrix when the tissue is heated. A peel test was applied on small samples of bovine longissimus epimysium to measure the transverse adhesion. The energy of peeling per surface is calculated. After 30 min of heating at 55°C, the energy of peeling was approximately the same as for the tissue in the raw state. It decreased by half between 60 and 65°C. After heating at 75°C, 25% of this energy still remained. These results show the destruction of the adhesive elements of the matrix with aqueous heating.

6.1.2 Introduction

The extracellular matrix plays an important role in the mechanical properties of raw connective tissues. It is made of proteoglycans, glycoproteins and of other molecules with adhesive properties. There are several types of proteoglycans which vary in amount among muscles [1]. During storage there is a decrease in the quantity of proteoglycans and consequently a decrease in raw perimysium strength [2]. In a study using eight pork muscles, a positive correlation of 0.49 was found between proteoglycan quantity and the shear force of raw meat [3]. A decrease in the strength of the perimysium of semitendinosus muscle from heifers during conditioning was observed for raw meat and in meat cooked at 50° C for 1hr, but not in meat cooked at higher temperatures [4].

For a better understanding of the sources of variations in meat tenderness, it is necessary to know accurately what happens to the matrix compounds during heating. Very little is known about their

thermal stability [4]. The denaturation of these compounds during heating will affect the force of adhesion between the extracellular matrix and collagen fibers. The purpose of this work was to quantify changes in adhesion between collagen fibers and the matrix as a result of aqueous heating.

6.1.3 Materials and Methods

Epimysium sheets were removed at 48 hr postmortem from the longissimus dorsi of 2 cows (6 and 7 years old) and were frozen at -20°C. Before use they were thawed at room temperature. Samples of approximate dimensions, length=100 mm, depth=15 mm and thickness=5 mm, were cut, with the longest dimension in the direction of collagen fibers, i.e., along the longitudinal axis of the longissimus dorsi.

Samples were selected randomly and subjected to thermal treatments (30 min in a water bath at temperatures of 55, 60, 65, or 75°C). During heating samples were free to contract and swell and the final dimensions of the samples after heating were measured with a micrometer. All the mean values presented were obtained from 7 to 10 samples.

In preparation for the peel test, a cut of 20 mm length was done at one end of the sample giving a Y shape. The two parts on either sides of the cut are used to grip the samples. The samples were extended using a TMS Pro texture system (Food Technology Corp., Sterling, Virginia, USA) at a rate of 200 mm/ min until complete separation.

From the force–distance curves (Figure 1) the breaking energy was calculated. The first part of the diagram is due to the extension of the fibers and is removed from the calculation of the energy. Using the thickness and length of the sample after heating the energy is expressed in mJ/mm^2 and corresponds to the energy per unit of surface area created during the test.

6.1.4 Results and Discussion

The peel test has been used by various food and non-food industries to measure the interfacial adhesion between various materials. This work illustrates its utility during the tearing of bovine connective tissue to document changes in adhesive force following aqueous heating.

At the beginning of the peel test there is a phase where collagen fibers are progressively stretched producing a continuous increase in stress without a break in the fibers (Figure 1, at displacements below 8 mm). The amplitude of this phase increases with temperature because

collagen fibers become more elastic. Then at a certain level of stress the samples begin to break, and show successive breaks (Figure 2, region between the dotted lines).

As shown in Figure 2, the peeling energy does not significantly change between raw samples and those heated at 55°C for 30 min. At heating temperatures above 55°C, the peeling energy deceases rapidly. Following 75°C heating, about 25% of the initial energy remains.

The peeling energy represents the energy necessary to create two surfaces, i.e., to separate the sample in two. There are several compounds involved in adhesion of the matrix to collagen fibers which have their own temperatures of denaturation and kinetics. Figure 2 shows that these compounds become progressively weaker with temperature but are still present at 80°C.

The breakage of molecules of adhesion in the tissue due to thermal treatment may be explained either as a simple denaturation process or as a mechanical stress. As a matter of fact, a stress-induced breakage of molecules of adhesion during heating must be considered as epimysium tissue swells and contracts with heating which modifies the distance between collagen fibers both in the longitudinal and transverse directions.

6.1.5 Conclusion

In a tissue with well oriented fibers such as epimysium, the adhesion between fibers can be easily quantified by a peel test. This work has shown that matrix modifications occur above 55°C and continue until 75°C. For rapidly cooked meat for which the core temperature is around 60°C, the contribution of the extracellular matrix to texture must be considered.

6.1.6 References

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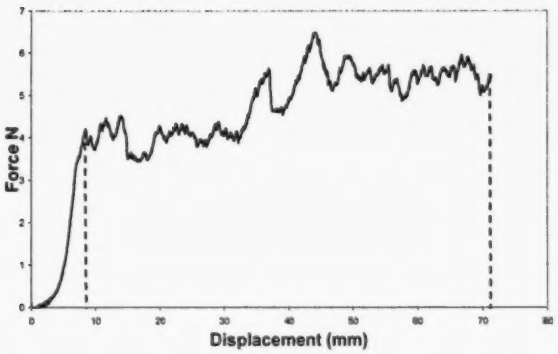

Figure 1: Force displacement curves from the peel test of an epimysium sheet. The peeling energy is calculated as the area under the curve and between the two dotted lines.

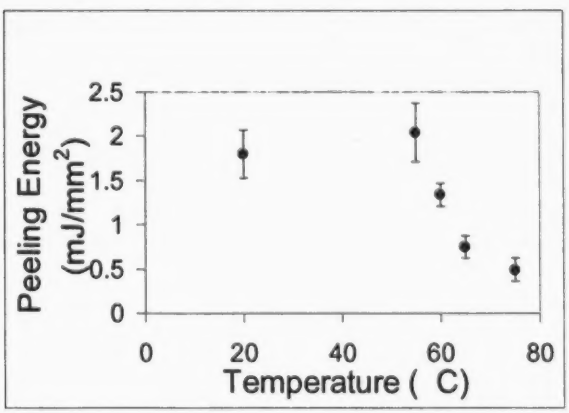

Figure 2: Variation of the peeling energy with temperature following heating in a water bath for 30 min.

Publication

Lepetit, J. and **Shand P.J.** 2009. Effect of heating on the transverse adhesion of collagen fibers. Proceedings of the 55th International Congress of Meat Science and Technology, Copenhagen, Denmark, August 16-21. PE7.15 pp. 1138-1141. [short paper and poster]